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Eco-Friendly Synthesis of Biologically Active ZnO Nanoparticles from Ouret Lanata

Poornima N.[1], Dr. K. Anbarasi[2]
[1]*MSc Chemistry, Department of Chemistry, Nirmala College for Women, Coimbatore-641018*
[2]*Associate Professor, Department of Chemistry, Nirmala College for Women, Coimbatore-641018*

Abstract: ***Green nano synthesis refers to the eco-friendly production of nanoparticles using biological resources instead of hazardous chemicals and energy-intensive processes. This approach follows the principles of green chemistry by reducing toxic reagents, minimizing waste generation, and lowering environmental impact. Green synthesis has gained significant attention due to increasing concerns about the safety and sustainability of conventional physical and chemical nanoparticle synthesis methods. Zinc oxide (ZnO) nanoparticles were successfully synthesized using an eco-friendly green synthesis method with Ouret lanata leaf extract as a natural reducing and stabilizing agent. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, FTIR, XRD, and SEM to study their optical, structural, and morphological properties. UV–Visible analysis showed strong absorption in the UV region, confirming the formation of ZnO nanoparticles. FTIR spectra revealed characteristic Zn–O stretching vibrations along with plant-derived functional groups responsible for stabilization. XRD analysis confirmed the crystalline hexagonal wurtzite structure of ZnO nanoparticles with high purity. SEM images showed nearly spherical to irregular-shaped nanoparticles with slight agglomeration. The biological activities of the ZnO nanoparticles were evaluated through antioxidant and antibacterial assays. The nanoparticles exhibited concentration-dependent antioxidant activity and significant antibacterial activity against Staphylococcus aureus and Escherichia coli. These findings suggest that green-synthesized ZnO nanoparticles possess promising biological properties and have potential applications in biomedical and pharmaceutical fields.***

Keywords: Green nano synthesis, sustainability, SEM, XRD analysis, Antioxidant activity, Antibacterial activity.

I. INTRODUCTION

Nanotechnology has emerged as a rapidly advancing field due to the unique physicochemical properties of materials at the nanoscale, such as high surface area, enhanced reactivity, and tunable optical and electronic characteristics. Among metal oxide nanoparticles, zinc oxide (ZnO) has attracted considerable attention because of its wide band gap, high exciton binding energy, chemical stability, non-toxicity, and cost-effectiveness. These properties make ZnO nanoparticles suitable for diverse applications including corrosion inhibition, photocatalysis, antimicrobial activity, sensors, cosmetics, and biomedical fields. Conventional methods for synthesizing ZnO nanoparticles, such as sol–gel, hydrothermal, chemical precipitation, and vapor deposition techniques, often involve toxic chemicals, high energy consumption, and environmentally hazardous by-products. These limitations have prompted the development of eco-friendly and sustainable synthesis routes. In this context, green synthesis has gained significant importance as it aligns with the principles of green chemistry by minimizing the use of harmful reagents and reducing environmental impact. Green synthesis of ZnO nanoparticles typically employs biological resources such as plant extracts, bacteria, fungi, and algae as reducing, stabilizing, and capping agents. Plant-mediated synthesis is particularly advantageous due to its simplicity, scalability, low cost, and the presence of natural phytochemicals such as flavonoids, phenolics, alkaloids, and proteins. These biomolecules play a crucial role in controlling particle size, morphology, and stability of the synthesized ZnO nanoparticles. The green synthesized ZnO nanoparticles often exhibit enhanced functional properties compared to their chemically synthesized counterparts, owing to the surface functionalization by bioactive compounds. This makes them especially attractive for environmentally benign applications such as green corrosion inhibitors, antimicrobial coatings, and sustainable nanocomposites. Thus, green nanosynthesis of ZnO represents a promising and sustainable approach for producing functional nanomaterials while addressing environmental and health concerns associated with traditional synthesis methods. Nanoparticles have surfaced as a flexible and promising category of materials possessing distinctive characteristics that can be utilized for multiple applications. The continuous investigation of green synthesis employing natural resources and biologically active substances for nanoparticle production aims to enhance processes, minimize environmental harm, and satisfy the growing demand for these nanostructures' applications.

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The use of biological resources allows for the cost-effective and quick synthesis of nanoparticles, regarded as a one-step process that maintains or enhances their physical and chemical characteristics. Thanks to the significant capabilities of this approach and the eco-friendly and effective generation of nanoparticles, various sizes and forms can be achieved, making it a highly appealing choice not only for nanostructure synthesis but also for using this method in the creation of other substances.The present study investigates the synthesis and biological applications of zinc oxide nano particle from *Ouret lanata* leaf extract using UV analysis, FTIR study, XRD , SEM analysis , anti bacterial and antioxidant studies.[1-10]

II. EXPERIMENTAL

A. Materials

1) Preparation of Ouret lanata extract

Ouret lanata(OL) leaves were collected from Kadiyangad, Kozhikode, Kerala. The fresh leaves of *Ouret lanata* were washed with distilled water and dried under the shade. After drying, the leaves were made into fine powder using mechanical blender and transferred into an air tight container. Accurately 25 g of powdered *Ouret lanata* leaves were weighed out and added to 500 ml distilled water in 400 ml beaker and heated for about 15-20 minutes. The solution is kept aside for settle and filtered.

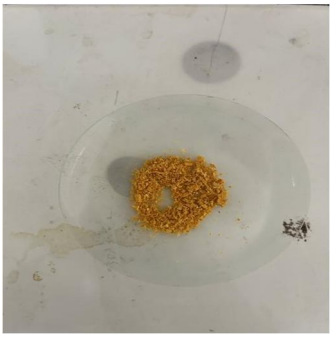

Dried Ouret lanata leaves


Ouret lanata leaf extract

2) Green synthesis of Zinc Oxide nanoparticles

About 3g of Zn $(NO_3)_2$.6H20 was dissolved in 25 ml of Plant extract with constant stirring.Then the mixture was placed in a magnetic stirrer at ambient temperature for one hour, until a precipitate is formed.The formation precipitate indicate the formation of Zinc oxide nano particle. The solution was dried and used for the characterization of Zinc oxide nano particle.


Preparation of ZnO NPs


Zinc oxide nanoparticle

B. Methods

1) Characterization Techniques

Various analytical techniques were used for the characterization of ZnO NPs in order to determine their size, functional groups, shape and crystallographic structure.These techniques include UV-Vis spectroscopy, FTIR, SEM and XRD.The optical properties of biosynthesized ZnO-NPs were studied using a UV-Vis spectrophotometer in the wavelength range of 300–800nm.The FTIR spectrophotometer was used for the identification of functional groups present in biosynthesized ZnO NPs within the range of 500-4000cm^{-1}.The crystalline structure of ZnO NPs was determined using an X-ray diffractometer.The SEM analysis was performed to determination of the morphology and size of ZnO NPs. The Characterizations was done at Avinashilingam Institute of Home Science and Higher Education for Women (Bharat Ratna prof. CNR Rao research Centre), Coimbatore.

2) Biological Activity

a) Anti oxidant assay

To evaluate the antioxidant potential, 0.12 mg of DPPH was accurately weighed and dissolved in 85 mL of pure methanol. The prepared DPPH solution was protected from light by wrapping the reagent bottle with aluminum foil and kept in a dark place for 1 h. As a standard control, ascorbic acid solution was prepared by dissolving 1 mg of ascorbic acid in 100 mL of distilled water. ZnO nanoparticle samples were prepared at concentrations of 10, 20, 30, 40, and 50 µg/mL. To each Eppendorf tube containing ZnO NPs, 800 µg/mL of DPPH solution was added, and the final volume was adjusted to 1.5 mL using methanol. The mixtures were then incubated in the dark for 1 h, during which a visible color change from purple/violet to yellow indicated free radical scavenging activity. The absorbance of each sample was recorded at 517 nm using a UV–Visible spectrophotometer, and the antioxidant activity of ZnO NPs was compared with the standard ascorbic acid. The percentage scavenging activity was calculated using the formula:

Scavenging Activity (%) = (Acontrol − Asample) / Acontrol × 100.

The anti-oxidant assay was done at Avinashilingam Institute of Home Science and Higher Education for Women (Bharat Ratna prof. CNR Rao research Centre), Coimbatore.

b) Anti microbial activity

An antibacterial study of ZnO NPs from leaf extract was performed using theagar-well diffusion method against Staphylococcus aureus and Escherichia coli. The antimicrobials present in the test samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. Petriplates containing 20 ml Mueller Hinton medium were seeded with 0.5 McFarland standard cultures of bacterial strains. Wells were cut using sterile well puncture and 50 µl of samples were were added into the wells.Chloramphenicol is used as the positive control.The plates were then incubated at 37°C for 24 hours.The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Anti microbial activity was performed at Avinashilingam Institute of Home Science and Higher Education for Women (Microbiology research centre), Coimbatore.

III. RESULTS AND DISCUSSION

A. UV-Visible Spectroscopy

The UV-visible spectra of synthesised ZnO NPs are shown in figure 1.A sharp increase in absorbance in the UV region is observed, particularly at the 220 nm (OD = 1.007), followed by moderate absorbance at 251 nm (OD = 0.067) and 292–290 nm (OD = 0.024–0.026)[11]. This strong UV absorption is attributed to intrinsic band-gap absorption arising from electron transitions from the valence band to the conduction band in ZnO. The steep absorption edge in the UV region confirms the nanoscale nature of ZnO particles. The absence of absorption peaks in the visible region suggests that the particles are well-formed, non-agglomerated, and of high purity.

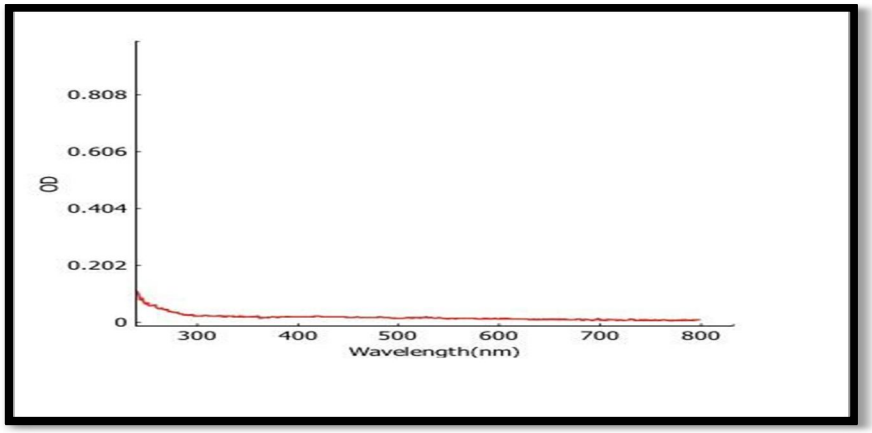


Figure 1. UV-Visible Spectroscopy of ZnO

B. FT-IR Analysis

The FTIR spectrum of the OL-Zn sample given in figure 2 and table 1 exhibits several characteristic peaks that confirm the synthesis of zinc oxide nanoparticles and the presence of organic stabilizing agents. The broad, intense peak at 3319.836 cm^{-1} is attributed to the O-H stretching vibration, which corresponds to the fundamental mode of hydroxyl groups from either adsorbed water or phenolic compounds within the extract. A sharp peak at 1635.477 cm^{-1} indicates the N-H bend or C=O stretching, suggesting that proteins or flavonoids from the "OL" extract are involved in the capping and stabilization process. The absorption band at 1103.415 cm^{-1} is assigned to C-N symmetric stretching or C-O vibrations, typical of the alcohol, ether, or ester functional groups identified in your reference model. Most importantly, the strong peak observed at the lower frequency of 435.209 cm^{-1} represents the characteristic Zn-O stretching vibration, which definitively confirms the formation of the ZnO nanoparticle structure.[12][13][14]

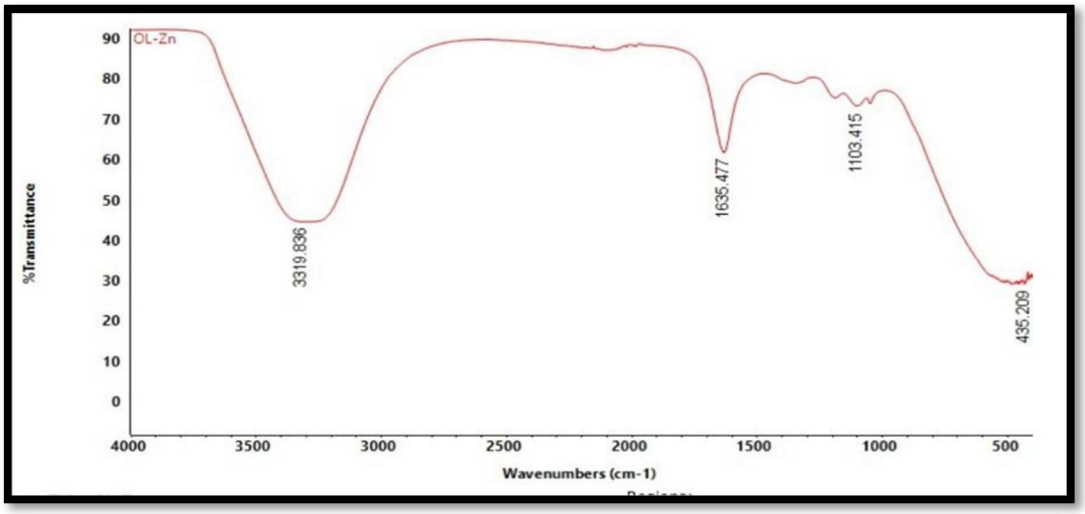


Figure 2. FTIR Analysis of ZnO

Table 1.FTIR Peak of ZnO

Peak value	Possible groups
3319.836 cm^{-1}	O-H stretching
1635.477 cm^{-1}	N-H or C=O stretching
1103.415 cm^{-1}	C-N or C-O stretching
435.209 cm^{-1}	Zn-O stretching

C. XRD Analysis

The X-ray diffraction (XRD) analysis of ZnO nanoparticles given in figure 3 and the peak values are listed in Table 2 , reveals their crystalline nature. The diffraction peaks observed at31.5^0-32^0,34^0-35^0,36^0-37^0,44^0,54^0-56^0,58^0-63^0corresponds to characteristic reflection of Zinc oxide nanoparticle.

These peaks can be indexed to the crystal planes (100), (002), (101), (102), (110), (103), (200), (112), and (201) respectively, which correspond well with the hexagonal wurtzite structure of ZnO. The presence of the characteristic and intense peak near 36^0-37^0, corresponding to the (101) plane, is a strong indication of ZnO formation[15]. This peak is considered the most intense reflection of hexagonal ZnO and confirms the conversion of zinc precursor into ZnO[16][17]. No additional impurity peaks related to zinc hydroxide, zinc nitrate, or other zinc-related phases are observed, indicating high purity of the synthesized ZnO nanoparticles. The calculated Full Width at Half Maximum (FWHM) values suggests nanoscale crystallite sizes, confirming the successful synthesis of ZnO in nanocrystalline form.The d-spacing values, ranging from 8.05Å to 1.56Å, further support the presence of a well-structured crystalline phase. The sharp and intense peaks indicate minimal amorphous content, reinforcing the purity of the synthesized nanoparticles. Overall, the XRD results confirm that the green synthesis method using *Ouret lanata* effectively produces crystalline ZnO nanoparticles with a well-defined hexagonal wurtzite structure.

Table 2. XRD Peak of ZnO

Position [^{0}2θ]	FWHM Left [^{0}2θ]	d-spacing [A^0]	Relative Intensity [%]
10.9890	0.4015	8.05152	55.44
13.7782	0.4015	6.42726	57.08
16.6869	0.4015	5.31289	30.55
19.0796	0.4015	4.65168	34.98
25.1679	0.4015	3.53853	54.13
29.0295	0.2676	3.07601	100.00
30.1827	0.4015	2.96105	39.74
31.5963	0.2007	2.83173	32.19
37.2674	0.4684	2.41282	48.50
44.2231	0.5353	2.04812	48.01
54.3526	0.9368	1.68795	24.14
58.9162	0.8029	1.56762	17.78

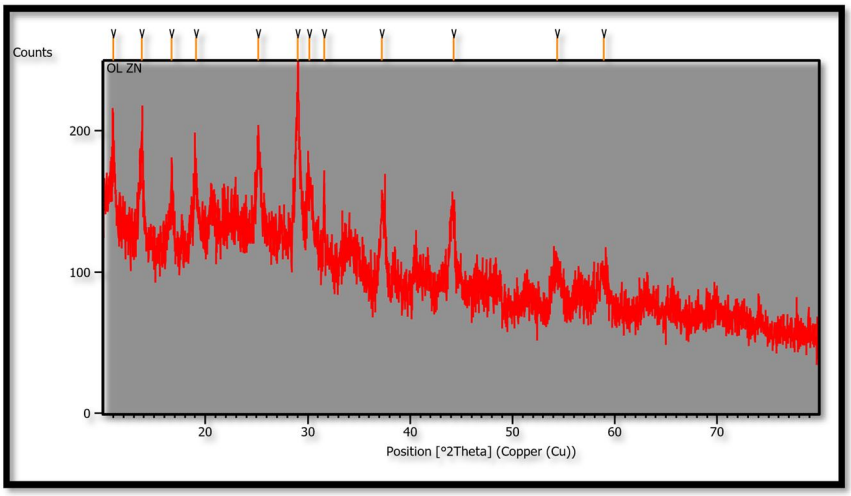


Figure 3. XRD pattern of ZnO

D. SEM Analysis

The SEM images of Zinc oxide (ZnO) nanoparticles synthesized using Ouret Lanata extract shown in figures [4a-4d] with different magnification images reveal essential characteristics of their surface morphology. The SEM image shows that the ZnO nanoparticles are predominantly nearly spherical to irregular in shape[18]. Slight variation in particle shape is commonly observed in green-synthesized nanoparticles due to the influence of plant biomolecules during nucleation and growth.The particles show a moderately uniform distribution, altpough some degree of agglomeration is visible. This agglomeration may be due to high surface energy of nanoparticles ,Presence of hydroxyl and organic functional groups on the surface ,drying process during sample preparation.Such agglomeration is typical for ZnO nanoparticles synthesized via green routes. The surface of the nanoparticles appears rough and porous, which increases the surface area. This rough texture is advantageous for applications such as antioxidant, antibacterial, photocatalytic, and sensor applications, as higher surface area enhances activity. The observed clustering and surface coating indicate the presence of organic compounds from the OL extract, which act as capping and stabilizing agents. These biomolecules prevent excessive particle growth and help maintain nanoscale size, though they may also cause partial aggregation. Overall, the SEM results confirm the successful green synthesis of ZnO nanoparticles and support their potential for various applications[19][20].

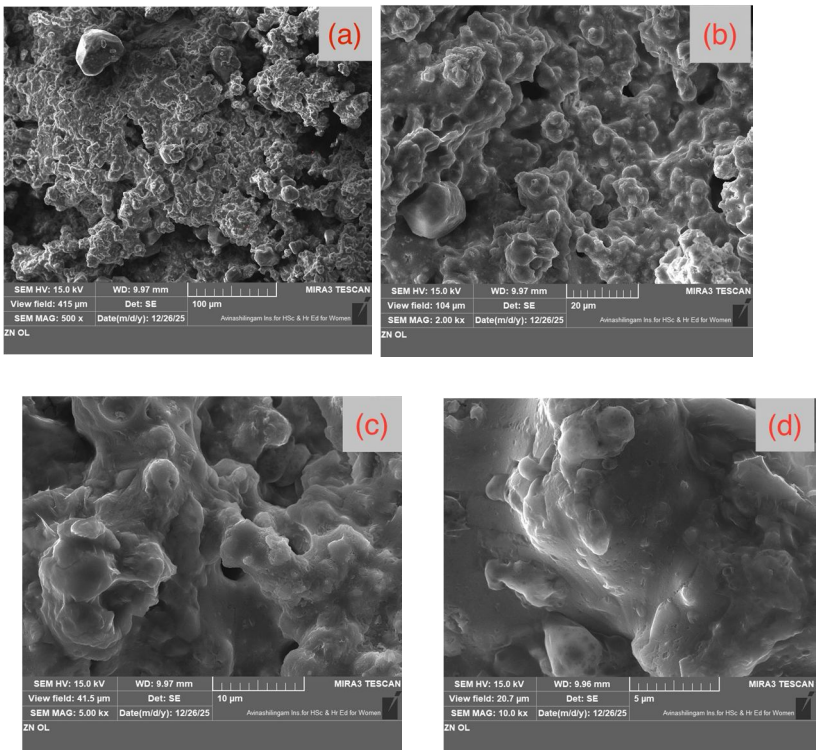


Figure 4(a-d).SEM images of green synthesised ZnO NPs at various magnification range

E. Biological Activity

1) Anti-oxidant Assay

The antioxidant activity of the synthesized sample Zn–OL along with the standard is give in table 3&4 and the percentage inhibition with increase in concentration is give in figure 5.Ascorbic acid exhibited a strong and concentration-dependent antioxidant activity. The percentage inhibition increased from 39.88% at 10 µg/ml to 73.61% at 14 µg/ml, demonstrating its high free radical scavenging efficiency. The standard calibration curve showed good linearity with a regression coefficient ($R^2 = 0.964$), confirming the reliability of the assay[21].

Table 3. Standard Ascorbic acid

Concentration(μ g/mL)	% Inhibition
10	39.88
11	51.38
12	58.77
13	65.27
14	73.61

Table 4. % Inhibition of Zn-OL

Concentration(μ /I)	% Inhibition
200	47.22
400	51.38
600	56.94
800	59.72
1000	66.66

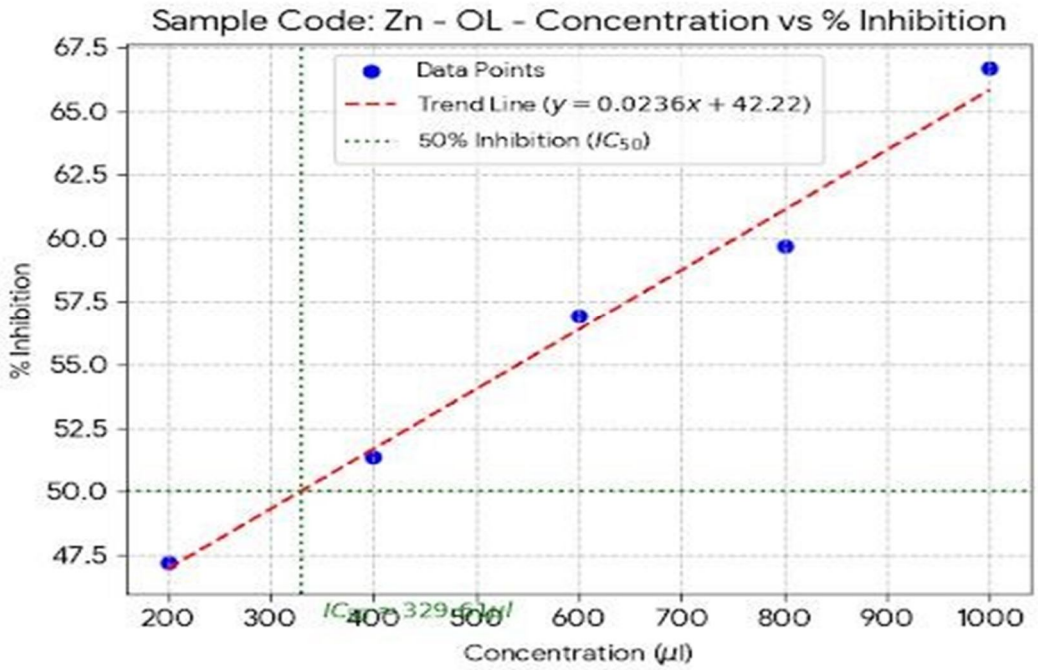


Figure 5. Graph of sample Zn-OL

The Zn–OL sample also showed a gradual increase in antioxidant activity with increasing concentration. The percentage inhibition was observed to be 47.22% at 200 µl, which increased to 51.38% at 400 µl, 56.94% at 600 µl, 59.72% at 800 µl, and reached a maximum of 66.66% at 1000 µl. This concentration-dependent trend indicates effective interaction of the sample with DPPH free radicals. The DPPH assay is based on the reduction of the purple-colored DPPH radical to a yellow-colored diphenylpicrylhydrazine in the presence of antioxidant molecules capable of donating hydrogen atoms or electrons. The observed increase in % inhibition with concentration for Zn–OL confirms its ability to scavenge free radicals. The results suggest that Zn–OL can effectively reduce oxidative stress by neutralizing free radicals, thereby indicating its potential application as a natural antioxidant material[22]. The concentration-dependent behavior further supports its suitability for biomedical and pharmaceutical applications.

2) Anti-bacterial Activity

The ZnOL sample exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria[23]. Notably, the zone of inhibition produced by ZnOL against Staphylococcus aureus was comparable to that of the standard antibiotic, indicating high effectiveness against Gram-positive bacteria. Against Escherichia coli, ZnOL showed a slightly higher zone of inhibition than the standard antibiotic, suggesting enhanced activity toward Gram-negative bacteria[24][25].

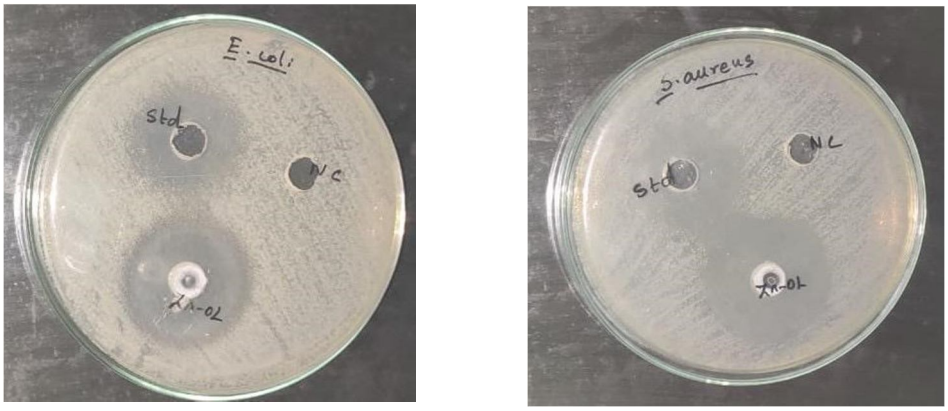


Figure 6.Anti bacterial activity ofZnO nanoparticle against Streptococcus aureus Escherichia coli

Table 5. Zone of inhibition of ZnO NP against *Streptococcus aureus* and *Escherichia coli*

Samples	Zone of inhibition in diameter (mm)	
	Staphylococcus aureus	*Escherichia coli*
Standard antibiotic (Positive control)	36.6±0.52	30.4±0.69
ZnOL – 50 µl	35.7±0.64	32.6±1.52

IV. CONCLUSION

In this study, ZNPs was synthesized by *Ouret lanata* leaves aqueous extract using zinc nitrate precursor.UV Visible spectrum showed a distinct peak around 290 nm, which is specific for ZNPs. The XRD results confirmed the efficiency of the synthesis process, evidencing the production of single crystalline ZNPs with hexagonal wurtzite structure. The average size of ZNPs synthesized by zinc nitrate was found to be 23.4 nm, exhibiting bullets and spherical like structures, respectively which were confirmed by XRD and SEM analyses. FT-IR studies clearly showed the formation of ZnO and indicated that the plant extract contains various phytochemicals, which work as capping and stabilizing agent for the synthesized ZNPs. The comparable or superior performance of ZnOL relative to the standard antibiotic highlights its potential as an effective antimicrobial agent, supporting its possible application in biomedical and pharmaceutical fields. From the analyses of results, it is clear that the precursor played a vital role in surface morphology and structure of ZNPs. Our results confirm the potential of *Ouret lanata* for the synthesis of ZNPs in a simple, fast and ecofriendly way.

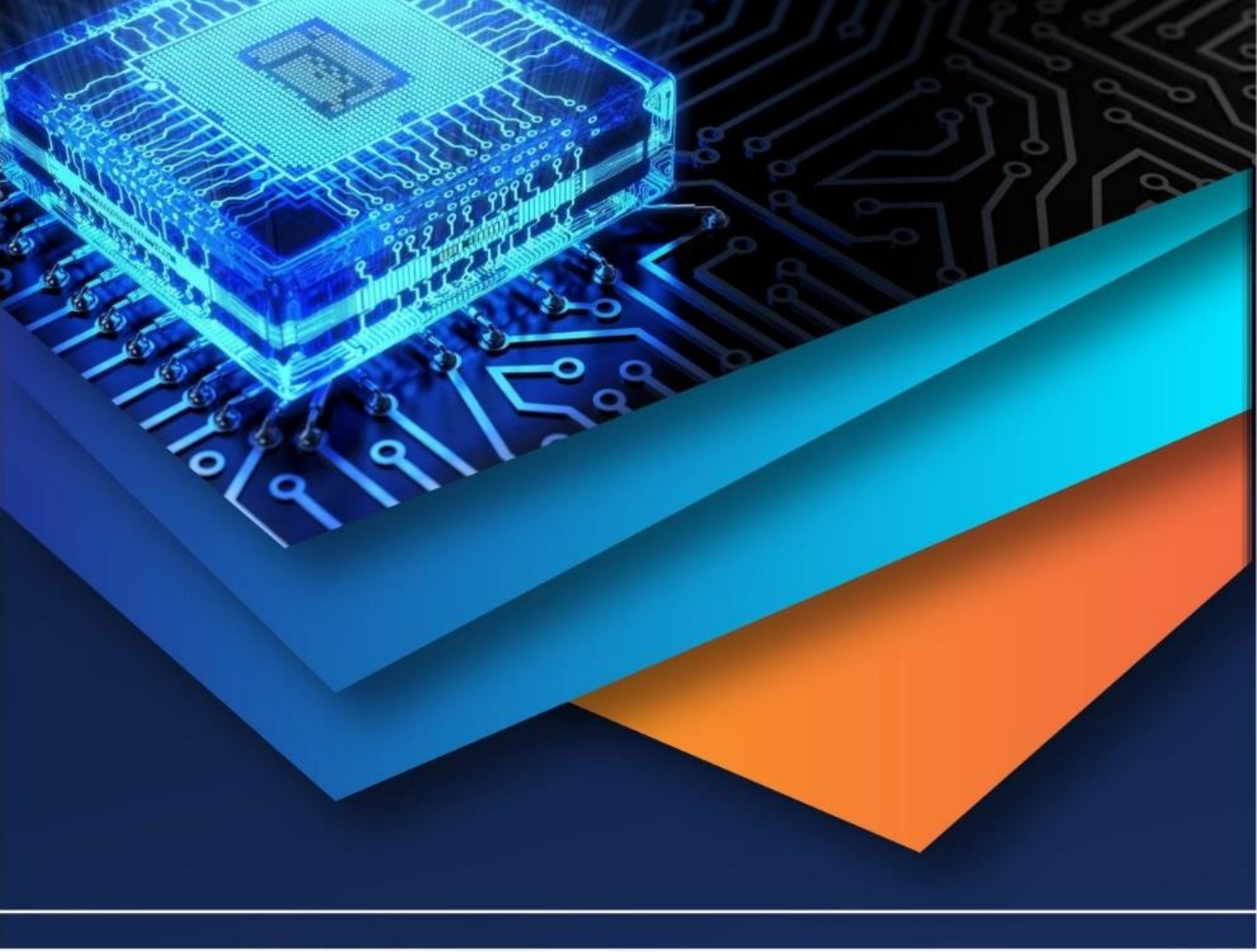
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